

Genome sequence of the squalene-degrading bacterium *Corynebacterium terpenotabidum* type strain Y-11^T (= DSM 44721^T)

Christian Rückert¹, Andreas Albersmeier¹, Arwa Al-Dilaimi¹, Hanna Bednarz², Karsten Niehaus², Rafael Szczepanowski¹, Jörn Kalinowski^{1*}

¹ Technology Platform Genomics, CeBiTec, Bielefeld University, Bielefeld, Germany

² Proteome and Metabolome Research, Bielefeld University, Bielefeld, Germany

*Correspondence: Jörn Kalinowski (Joern@CeBiTec.Uni-Bielefeld.DE)

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Corynebacterium terpenotabidum Takeuchi *et. al* 1999 is a member of the genus *Corynebacterium*, which contains Gram-positive and non-spore forming bacteria with a high G+C content. *C. terpenotabidum* was isolated from soil based on its ability to degrade squalene and belongs to the aerobic and non-hemolytic *Corynebacteria*. It displays tolerance to salts (up to 8%) and is related to *Corynebacterium variabile* involved in cheese ripening. As this is a type strain of *Corynebacterium*, this project describing the 2.75 Mbp long chromosome with its 2,369 protein-coding and 72 RNA genes will aid the *Genomic Encyclopedia of Bacteria and Archaea* project.

Introduction

Strain Y-11^T (= DSM 44721^T) is the type strain of the species *Corynebacterium terpenotabidum* [1]. It was originally isolated from soil, although the exact source has not been published [2,3]. The genus *Corynebacterium* is comprised of Gram-positive bacteria with a high G+C content. It currently contains over 80 members [4] isolated from diverse backgrounds like human clinical samples [5] and animals [6], but also from soil [7] and ripening cheese [8].

Within this diverse genus, *C. terpenotabidum* has been proposed to form a subclade together with *C. variabile* DSM 20132^T and *C. nuruki* S6-4^T, demonstrating 97.4% and 95.9% similarity respectively between the 16S rRNA gene sequences. Information on the strain is scarce. It was isolated for its ability to metabolize the linear triterpene squalene and classified as an *Arthrobacter* species [2,3], but no further information on the strain was supplied. Neither the origin nor the exact isolation procedures were reported. *C. terpenotabidum* can cleave squalene yielding geranylacetone [2] but also accepts some squalene derivatives [3].

Here we present a summary classification and a set of features for *C. terpenotabidum* DSM 44721^T,

together with the description of the genomic sequencing and annotation.

Classification and features

A representative genomic 16S rRNA sequence of *C. terpenotabidum* DSM 44721^T was compared to the Ribosomal Database Project database [9]. *C. terpenotabidum* shows highest similarity to *C. variabile* (97.4%).

Figure 1 shows the phylogenetic neighborhood of *C. terpenotabidum* in a 16S rRNA based tree. Within the genus *Corynebacterium*, *C. terpenotabidum* forms a distinct subclade together with *C. variabile* and *C. nuruki*.

C. terpenotabidum Y-11^T cells are Gram-positive non acid fast rods (1.0–1.5 µm x 0.5–0.8 µm wide) that grow strictly aerobically in rough, grayish-white colonies without diffusible pigments or aerial mycelia [1], [Table 1]. Cells grow with a wax-like quality on solid medium and tend to clot in liquid culture. Scanning electron micrograph pictures of liquid grown cultures revealed slight morphological differences between free-floating cells and clotted cells (Figure 2).

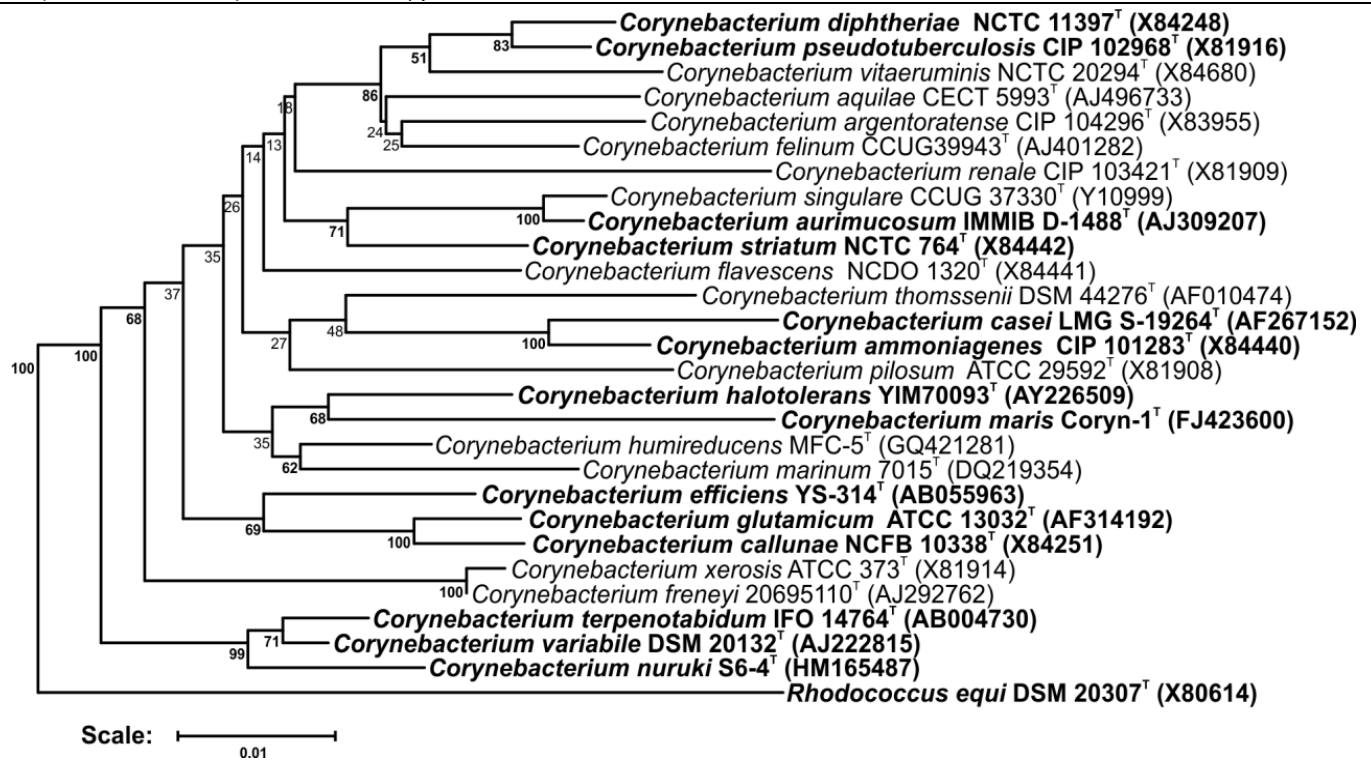


Figure 1. Phylogenetic tree highlighting the position of *C. terpenotabidum* relative to type strains of other species within the genus *Corynebacterium*. Species with at least one publicly available genome sequence (not necessarily the type strain) are highlighted in **bold face**. The tree is based on sequences aligned by the RDP aligner and utilizes the Jukes-Cantor corrected distance model to construct a distance matrix based on alignment model positions without alignment inserts, using a minimum comparable position of 200. The tree is built with RDP Tree Builder, which utilizes the Weighbor method [10] with an alphabet size of 4 and length size of 1,000. The building of the tree also involves a bootstrapping process repeated 100 times to generate a majority consensus tree [11]. *Rhodococcus equi* (X80614) was used as an outgroup.

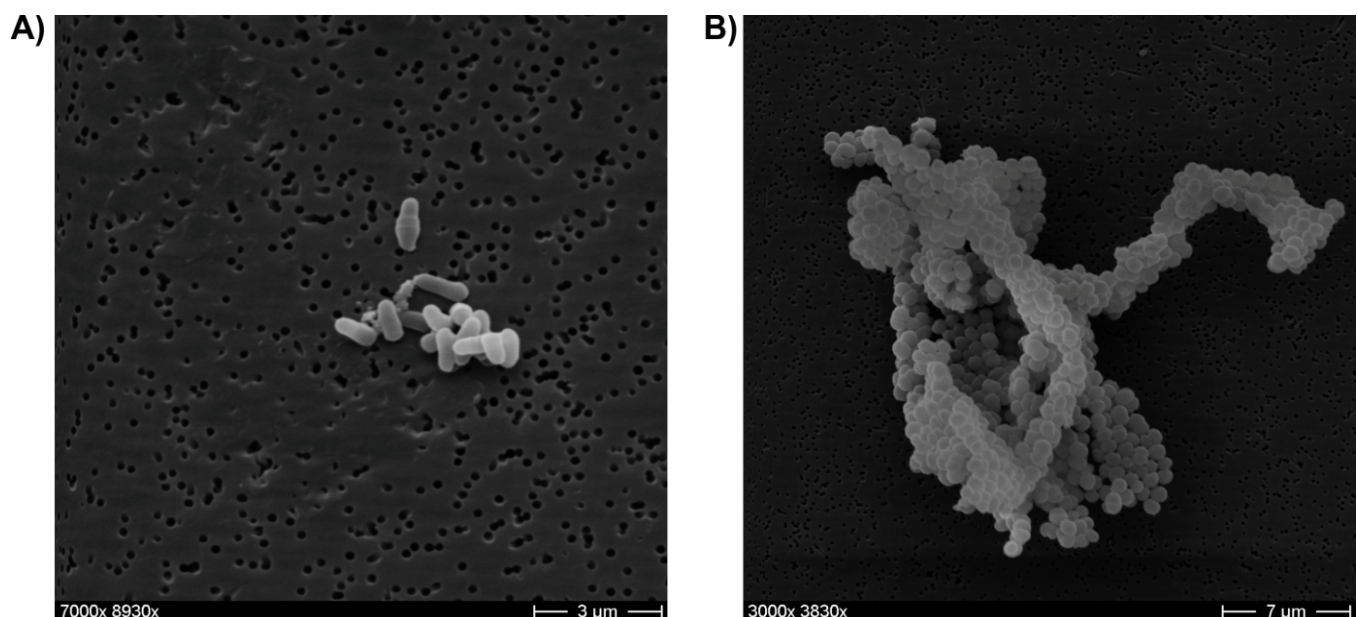


Figure 2. Scanning electron micrograph of *C. terpenotabidum* Y-11^T. A) Free-floating cells. B) Aggregated cells.

Table 1. Classification and general features of *C. terpenotabidum* Y-11^T according to the MIGS recommendations [12].

MIGS ID	Property	Term	Evidence code
		Domain <i>Bacteria</i>	TAS [13]
		Phylum <i>Actinobacteria</i>	TAS [14]
		Class <i>Actinobacteria</i>	TAS [15]
		Order <i>Actinomycetales</i>	TAS [15-18]
	Current classification	Family <i>Corynebacteriaceae</i>	TAS [15-17,19]
		Genus <i>Corynebacterium</i>	TAS [15-17,20,21]
		Species <i>Corynebacterium terpenotabidum</i>	TAS [1]
		Type-strain Y-11 (=DSM 44721)	TAS [1]
	Gram stain	positive	TAS [1]
	Cell shape	rod-shaped	TAS [1]
	Motility	non-motile	TAS [1]
	Sporulation	non-sporulating	TAS [1]
	Temperature range	mesophile	TAS [1]
	Optimum temperature	28°C	TAS [1]
	Salinity	0-8% (w/v) NaCl	TAS [1]
MIGS-22	Oxygen requirement	aerobe	TAS [1]
	Carbon source	fructose, galactose, mannose, lactate, ethanol	TAS [1]
	Energy metabolism	chemoorganoheterotrophic	NAS
	Terminal electron acceptor	oxygen	NAS
MIGS-6	Habitat	soil	TAS [2]
MIGS-15	Biotic relationship	free-living	NAS
MIGS-14	Pathogenicity	non-pathogenic	NAS
	Biosafety level	1	NAS
MIGS-23.1	Isolation	not reported	
MIGS-4	Geographic location	not reported	
MIGS-5	Sample collection time	not reported	
MIGS-4.1	Latitude	not reported	
MIGS-4.2	Longitude		
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

a) Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [22].

C. terpenotabidum was found to be able to utilize fructose, galactose, mannose, lactate, and ethanol as carbon source, while many others like arginine, aspartate, histidine, methylamine, ethylamine, methanol, galactose, lactose, maltose, sucrose, glycerol, sorbitol, mannitol, inositol, citrate, succinate, malonate, pimelate, *m*-hydroxybenzoate and *p*-hydroxybenzoate cannot be used. Optimal growth of strain Y-11^T is reported at 28°C. *C. terpenotabidum* was shown to grow with a salinity

between 0 and 8.0% (w/v NaCl), with no growth at 10% [1]. The biochemical characterization revealed positive signals for urease, catalase, and hydrolysis of Tween 80.

Chemotaxonomy

The cell wall of *C. terpenotabidum* Y-11^T contains alanine, glutamic acid, and meso-diaminopimelic acid in a molar ratio of 2.12: 1.00: 0.97. The main components of the cell wall sugars are described

to be arabinose, galactose, and mannose in a molar ratio of 2.47: 1.71: 1.00. The glycan moiety of the cell wall was found to contain acetyl residues [1].

In *C. terpenotabidum*, cellular fatty acids are composed mainly of oleic acid (C_{18:1}ω₉, 31%), palmitic acid (C_{16:0}, 28%), and tuberculostearic acid 10-methyl (C_{18:0}, 21%). The whole-cell methanolysate of strain Y-11 contained mycolic esters [1]. The predominant isoprenoid quinone is menaquinone MK-9(H₂).

Genome sequencing and annotation

Genome project history

C. terpenotabidum Y-11^T was selected for sequencing as part of a project to define the core genome and pan genome of the non-pathogenic corynebacteria. While not being part of the *Genomic Encyclopedia of Bacteria and Archaea* (GEBA) project [23], sequencing of the type strain will nonetheless aid the GEBA effort. The genome project is deposited in the Genomes OnLine Database [24] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the Center of Biotechnology (CeBiTec). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Two genomic libraries: one 454 pyrosequencing PE library (3.4 kb insert sizes), one Illumina library
MIGS-29	Sequencing platforms	454 GS FLX Titanium, Illumina MiSeq
MIGS-31.2	Sequencing coverage	29.52× Pyrosequencing; 61.71 × SBS
MIGS-30	Assemblers	Newbler version 2.3
MIGS-32	Gene calling method	GeneMark, Glimmer
	INSDC ID	CP003696
	GenBank Date of Release	September 1, 2013 / after publication
	GOLD ID	Gi18852
	NCBI project ID	168617
MIGS-13	Source material identifier	DSM 44721
	Project relevance	Industrial, GEBA

Growth conditions and DNA isolation

C. terpenotabidum strain Y-11^T, DSM 44721, was grown aerobically in LB broth (Carl Roth GmbH, Karlsruhe, Germany) at 30 °C. DNA was isolated from ~ 10⁸ cells using the protocol described by Tauch *et al.* 1995 [25].

Genome sequencing and assembly

The genome was sequenced using a 454 sequencing platform. A standard 3k paired end sequencing library was prepared according to the manufacturers protocol (Roche). The genome was sequenced using the GS-FLX platform with Titanium chemistry, yielding 384,252 total reads, providing 29.52× coverage of the genome. Pyrosequencing reads were assembled using the Newbler assembler v2.3 (Roche). The initial Newbler assembly consisted of 22 contigs in six scaffolds. Analysis of the six scaffolds revealed five that made up the

chromosome, while the remaining one contained five copies of the RRN operon that caused the scaffold breaks. The scaffolds were ordered based on alignments to the complete genomes of *C. variabile* [26] and subsequent verification by restriction digestion, Southern blotting and hybridization with a 16S rDNA specific probe.

The Phred/Phrap/Consed software package [27-30] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, gaps between contigs were closed by editing in Consed (for repetitive elements) and by PCR with subsequent Sanger sequencing (IIT Biotech GmbH, Bielefeld, Germany). A total of 12 additional reactions were necessary to close gaps not caused by repetitive elements.

To raise the quality of the assembled sequence, Illumina reads were used to correct potential base errors and increase consensus quality. A WGS library was prepared using the Illumina-Compatible Nextera DNA Sample Prep Kit (Epicentre, WI, U.S.A) according to the manufacturer's protocol. The library was sequenced in a 2x 120 bp paired read run on the MiSeq platform, yielding 2,307,926 total reads. Together, the combination of the Illumina and 454 sequencing platforms provided 91.2× coverage of the genome.

Genome annotation

Gene prediction and annotation were done using the PGAAP pipeline [31]. Genes were identified using GeneMark [32], GLIMMER [33], and Prodigal [34]. For annotation, BLAST searches against the NCBI Protein Clusters Database [35] are performed and the annotation is enriched by searches against the Conserved Domain Database [36] and subsequent assignment of coding sequences to

COGs. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [37], Infernal [38], RNAMmer [39], Rfam [40], TMHMM [41], and SignalP [42].

Genome properties

The genome consists of one circular chromosome of 2,751,233 bp (67.02% G+C content) with no additional extrachromosomal elements present. A total of 2,441 genes were predicted, 2,369 of which are protein coding genes. 1,306 (55.13%) of the protein coding genes were assigned to a putative function with the remaining annotated as hypothetical proteins. In addition, 910 protein coding genes belong to 281 paralogous families in this genome, corresponding to a gene content redundancy of 38.41% [Figure 3]. The properties and the statistics of the genome are summarized in Table 3, and Table 4.

Table 3. Genome Statistics

Attribute	Value	% of total ^a
Genome size (bp)	2,751,233	100.00
DNA coding region (bp)	2,441,394	88.74
DNA G+C content (bp)	1,843,810	67.02
Total genes	2,441	100.00
RNA genes	72	2.96
rRNA operons	5	
tRNA genes	57	2.34
Protein-coding genes	2,369	97.04
Genes with function prediction (protein)	1,306	55.13
Genes assigned to COGs	1,812	74.23
Genes in paralog clusters	910	38.41
Genes with signal peptides	224	9.54
Genes with transmembrane helices	606	25.58

a) The total is based on either the size of the genome in base pairs or the total number of genes in the annotated genome.

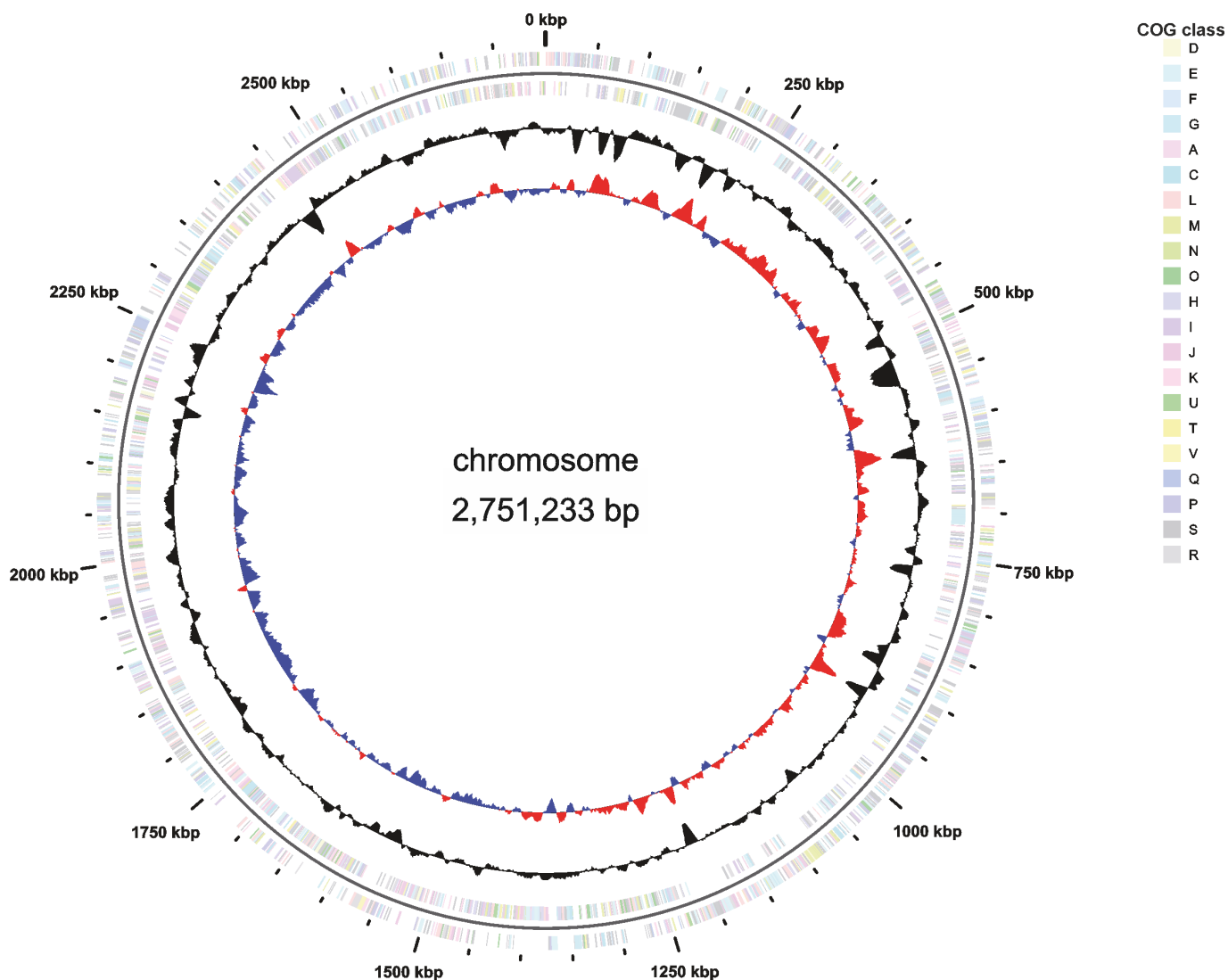


Figure 3. Graphical map of the chromosome. From the outside in: Genes on forward strand (colored according to COG categories), Genes on reverse strand (colored according to COG categories), GC content, GC skew.

Table 4. Number of genes associated with the general COG functional categories

Code	Value	%age	Description
J	151	6.37	Translation, ribosomal structure and biogenesis
A	1	0.04	RNA processing and modification
K	152	6.42	Transcription
L	136	5.74	Replication, recombination and repair
B	0	0.00	Chromatin structure and dynamics
D	20	0.84	Cell cycle control, cell division, chromosome partitioning
Y	0	0.00	Nuclear structure
V	32	1.35	Defense mechanisms
T	58	2.45	Signal transduction mechanisms
M	81	3.42	Cell wall/membrane biogenesis
N	1	0.04	Cell motility
Z	0	0.00	Cytoskeleton
W	0	0.00	Extracellular structures
U	26	1.10	Intracellular trafficking and secretion, and vesicular transport
O	72	3.04	Posttranslational modification, protein turnover, chaperones
C	127	5.36	Energy production and conversion
G	115	4.85	Carbohydrate transport and metabolism
E	218	9.20	Amino acid transport and metabolism
F	68	2.87	Nucleotide transport and metabolism
H	97	4.09	Coenzyme transport and metabolism
I	121	5.11	Lipid transport and metabolism
P	151	6.37	Inorganic ion transport and metabolism
Q	76	3.21	Secondary metabolites biosynthesis, transport and catabolism
R	274	11.57	General function prediction only
S	138	5.83	Function unknown
-	557	23.51	Not in COGs

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References

1. Takeuchi M, Sakane T, Nihira T, Yamada Y, Imai K. *Corynebacterium terpenotabidum* sp. nov., a bacterium capable of degrading squalene. *Int J Syst Bacteriol* 1999; **49**:223-229. [PubMed](#)
<http://dx.doi.org/10.1099/00207713-49-1-223>
2. Yamada Y, Motoi H, Kinoshita S, Takada N, Okada H. Oxidative degradation of squalene by *Arthrobacter* species. *Appl Microbiol* 1975; **29**:400-404. [PubMed](#)
3. Yamada Y, Kusuhara N, Okada H. Oxidation of linear terpenes and squalene variants by *Arthrobacter* sp. *Appl Environ Microbiol* 1977; **33**:771-776. [PubMed](#)
4. Euzéby JP. List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* 1997; **47**:590-592. [PubMed](#)
<http://dx.doi.org/10.1099/00207713-47-2-590>
5. Renaud FNR, Aubel D, Riegel P, Meugnier H, Bollet C. *Corynebacterium freneyi* sp. nov., alpha-

- glucosidase-positive strains related to *Corynebacterium xerosis*. *Int J Syst Evol Microbiol* 2001; **51**:1723-1728. [PubMed](#)
<http://dx.doi.org/10.1099/00207713-51-5-1723>
6. Collins MD, Hoyles L, Foster G, Falsen E. *Corynebacterium caspium* sp. nov., from a Caspian seal (*Phoca caspica*). *Int J Syst Evol Microbiol* 2004; **54**:925-928. [PubMed](#)
<http://dx.doi.org/10.1099/ijs.0.02950-0>
7. Zhou Z, Yuan M, Tang R, Chen M, Lin M, Zhang W. *Corynebacterium deserti* sp. nov., isolated from desert sand. *Int J Syst Evol Microbiol* 2012; **62**:791-794. [PubMed](#)
<http://dx.doi.org/10.1099/ijs.0.030429-0>
8. Brennan NM, Brown R, Goodfellow M, Ward AC, Beresford TP, Simpson PJ, Fox PF, Cogan TM. *Corynebacterium mooreparkense* sp. nov. and *Corynebacterium casei* sp. nov., isolated from the surface of a smear-ripened cheese. *Int J Syst Evol Microbiol* 2001; **51**:843-852. [PubMed](#)
<http://dx.doi.org/10.1099/00207713-51-3-843>
9. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 2009; **37**(Database issue):D141-D145. [PubMed](#)
<http://dx.doi.org/10.1093/nar/gkn879>
10. Bruno WJ, Socci ND, Halpern AL. Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. *Mol Biol Evol* 2000; **17**:189-197. [PubMed](#)
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026231>
11. Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrity GM, Tiedje JM. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Res* 2007; **35**(Database issue):D169-D172. [PubMed](#)
<http://dx.doi.org/10.1093/nar/gkl889>
12. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, et al. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; **26**:541-547. [PubMed](#)
<http://dx.doi.org/10.1038/nbt1360>
13. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 1990; **87**:4576-4579. [PubMed](#)
<http://dx.doi.org/10.1073/pnas.87.12.4576>
14. Garrity GM, Holt JG. The Road Map to the Manual. In: Garrity GM, Boone DR, Castenholz RW (eds), *Bergey's Manual of Systematic Bacteriology*, Second Edition, Volume 1, Springer, New York, 2001, p. 119-169.
15. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a New Hierarchic Classification System, *Actinobacteria* classis nov. *Int J Syst Bacteriol* 1997; **47**:479-491.
<http://dx.doi.org/10.1099/00207713-47-2-479>
16. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* 2009; **59**:589-608. [PubMed](#)
<http://dx.doi.org/10.1099/ijs.0.65780-0>
17. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. *Int J Syst Bacteriol* 1980; **30**:225-420.
<http://dx.doi.org/10.1099/00207713-30-1-225>
18. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the Schizomycetes. *J Bacteriol* 1917; **2**:155-164. [PubMed](#)
19. Lehmann KB, Neumann R. *Lehmann's Medizin, Handatanten. X Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik.*, Fourth Edition, Volume 2, J.F. Lehmann, München, 1907, p. 270.
20. Lehmann KB, Neumann R. *Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik*, First Edition, J.F. Lehmann, München, 1896, p. 1-448.
21. Bernard KA, Wiebe D, Burdz T, Reimer A, Ng B, Singh C, Schindle S, Pacheco AL. Assignment of *Brevibacterium stationis* (ZoBell and Upham 1944) Breed 1953 to the genus *Corynebacterium*, as *Corynebacterium stationis* comb. nov., and emended description of the genus *Corynebacterium* to include isolates that can alkalize citrate. *Int J Syst Evol Microbiol* 2010; **60**:874-879. [PubMed](#)
<http://dx.doi.org/10.1099/ijs.0.012641-0>
22. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**:25-29. [PubMed](#)
<http://dx.doi.org/10.1038/75556>

23. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, *et al.* A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*. *Nature* 2009; **462**:1056-1060. [PubMed](#) <http://dx.doi.org/10.1038/nature08656>
24. Liolios K, Chen IM, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, Kyrpides NC. The Genomes OnLine Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2010; **38**:D346-D354. [PubMed](#) <http://dx.doi.org/10.1093/nar/gkp848>
25. Tauch A, Kassing F, Kalinowski J, Pühler A. The *Corynebacterium xerosis* composite transposon Tn5432 consists of two identical insertion sequences, designated IS1249, flanking the erythromycin resistance gene *ermCX*. *Plasmid* 1995; **34**:119-131. [PubMed](#) <http://dx.doi.org/10.1006/plas.1995.9995>
26. Schröder J, Maus I, Trost E, Tauch A. Complete genome sequence of *Corynebacterium variabile* DSM 44702 isolated from the surface of smear-ripened cheeses and insights into cheese ripening and flavor generation. *BMC Genomics* 2011; **12**:545. [PubMed](#) <http://dx.doi.org/10.1186/1471-2164-12-545>
27. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res* 1998; **8**:175-185. [PubMed](#) <http://dx.doi.org/10.1101/gr.8.3.175>
28. Gordon D, Abajian C, Green P. Consed: a graphical tool for sequence finishing. *Genome Res* 1998; **8**:195-202. [PubMed](#) <http://dx.doi.org/10.1101/gr.8.3.195>
29. Gordon D. Viewing and editing assembled sequences using Consed. *Curr Protoc Bioinformatics* 2003;Chapter 11:Unit11 2.
30. Ewing B, Hillier L, Wendl MC, Green P. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res* 1998; **8**:175-185. [PubMed](#) <http://dx.doi.org/10.1101/gr.8.3.175>
31. NCBI. 2010 NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP). <http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>.
32. Borodovsky M, Mills R, Besemer J, Lomsadze A. Prokaryotic gene prediction using GeneMark and GeneMark.hmm. *Curr Protoc Bioinformatics* 2003;Chapter 4:Unit4 5.
33. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res* 1999; **27**:4636-4641. [PubMed](#) <http://dx.doi.org/10.1093/nar/27.23.4636>
34. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010; **11**:119. [PubMed](#) <http://dx.doi.org/10.1186/1471-2105-11-119>
35. Klimke W, Agarwala R, Badretin A, Chetvernin S, Ciufu S, Fedorov B, Kiryutin B, O'Neill K, Resch W, Resenchuk S, *et al.* The National Center for Biotechnology Information's Protein Clusters Database. *Nucleic Acids Res* 2009; **37**(Database issue):D216-D223. [PubMed](#) <http://dx.doi.org/10.1093/nar/gkn734>
36. Marchler-Bauer A, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, *et al.* CDD: specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res* 2009; **37**(Database issue):D205-D210. [PubMed](#) <http://dx.doi.org/10.1093/nar/gkn845>
37. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997; **25**:955-964. [PubMed](#)
38. Eddy SR. A memory-efficient dynamic programming algorithm for optimal alignment of a sequence to an RNA secondary structure. *BMC Bioinformatics* 2002; **3**:18. [PubMed](#) <http://dx.doi.org/10.1186/1471-2105-3-18>
39. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007; **35**:3100-3108. [PubMed](#) <http://dx.doi.org/10.1093/nar/gkm160>
40. Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A. Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* 2005;**33** (Database Issue):D121-124.
41. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 2001; **305**:567-580. [PubMed](#) <http://dx.doi.org/10.1006/jmbi.2000.4315>
42. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 2004; **340**:783-795. [PubMed](#) <http://dx.doi.org/10.1016/j.jmb.2004.05.028>